THE HYBRIDIZATION PROPERTIES OF 5′-END SEQUENCES OF GIANT NUCLEAR dRNA

A.P. RYSKOV, V.L. MANTIEVA, E.R. AVAKIAN and G.P. GEORGIEV
Institute of Molecular Biology, Academy of Sciences of the USSR, Moscow, USSR

Received 19 October 1970

1. Introduction

It has previously been shown [1] that giant dRNA contains triphosphate groups at the 5′-ends which may be considered as markers of the starting sequences of operons. In this paper hybridization analysis of the 5′-end sequences is reported. The hybridizability of triphosphorylated 5′-end sequences was found to be 3–4 times greater than that of whole dRNA molecules. Thus, the beginnings of the operons are enriched in repetitive base sequences, which is in good agreement with the previously presented model of operon structure in Eukaryotes [2].

2. Materials and methods

dRNA was isolated from rat livers, labelled *in vivo* with $Na_2H^{32}PO_4$ (1–2 mCi per animal) for 2–3 hr by hot phenol fractionation [3, 4] with slight modifications [1]. dRNA was ultracentrifuged in an SDS-sucrose gradient. The heavy fraction was collected and purified by additional DNase and pronase treatments, followed by phenol and chloroform deproteinization and by gel-filtration through Biogel P-100. The dRNA was then hybridized with an excess of homologous DNA (dRNA/DNA 1 : 50). The technique of DNA gels, cross-linked by UV radiation [9], was used to make it possible to work with large amounts of DNA. The mixtures of DNA gel fibres and dRNA in 2 × SSC were annealed for 14 hr at 65°. It was shown that in these conditions the loss of γ and β phosphates from nucleoside triphosphates comprises about 10–15%. The non-bound RNA was washed out by 2 × SSC at 65° four times. Then the gels were treated with RNase (1 mg RNase-free from traces of DNase per 20 mg DNA) and again washed by 2 × SSC four times at room temperature. Aliquots were taken from all supernatants and radioactivity was measured. The fractions containing the bulk of non-bound RNA were combined. The hybridized and non-hybridized materials were hydrolyzed by 0.5 N KOH and the hydrolysates were analysed for Xp, pXp, and pppXp content as was described previously [1]. The radioactivity of the fraction was determined in an "Intertechnique" scintillation counter.

3. Results

The results of two typical experiments are presented in table 1. In experiment (1) 3.7 mg of dRNA and 180 mg of DNA were taken, and in experiment (2) 2.0 mg of dRNA and 100 mg of DNA. The volumes were 50 ml and 30 ml respectively. One can see that the content of pppXp residues in hybridized material is about four times higher than in non-hybridized material. Thus the hybridizability of triphosphorylated 5′-end sequences in giant dRNA is much higher than that of whole molecules. On the other hand one can see that the contents of pXp in hybridized and non-hybridized material are of the same order although variable.

4. Discussion

It is known that the hybridizability of dRNA under our conditions indicates what part of the given RNA has been synthesized on highly repetitive DNA base sequences [5–8]. Thus one can conclude that the starting sequences in operons are represented by multiple base sequences. This result is in good agreement with the hypothesis on the operon structure in Eukaryotes described earlier [2]. According to the latter,

Table 1
The hybridizability of 5′-ends in heavy dRNA precursor molecules.

Experiment	Hybridization of total RNA, %	RNA fraction	Xp, cpm × 10^{-6}	pppXp		pXp	
				cpm	percent of total activity	cpm	percent of total activity
1	11	Hybridized	0.11	130	0.118	30	0.027
		Non-hybridized *	0.52	140	0.027	110	0.021
2	7	Hybridized	0.17	108	0.062	61	0.035
		Non-hybridized *	0.39	68	0.018	272	0.07

* Only part of the non-hybridized material was analysed.

many identical repetitive DNA base sequences are localized in promoter proximal parts of different operons. They correspond to the multiple acceptor sites. Regulatory agents switch on or off a great number of operons simultaneously by interaction with these sites.

Another conclusion from the above results is that the monophosphorylated 5′-ends are not formed from triphosphorylated ends by means of dephosphorylation but produced as a result of nuclease attack during the processing of giant dRNA. This follows from the marked difference in hybridizability of pppXp- and pXp-containing 5′-ends.

References

[1] A.P. Ryskov and G.P. Georgiev, FEBS Letters 8 (1970) 186.
[2] G.P. Georgiev, J. Theoret. Biol. 25 (1969) 473.
[3] G.P. Georgiev and V.L. Mantieva, Biokhimiya 27 (1962) 949.
[4] V.Ya. Arion, V.L. Mantieva and G.P. Georgiev, Molekul. Biol. 1 (1967) 689.
[5] R.J. Britten and D.E. Kohne, Science 161 (1968) 529.
[6] L.N. Ananieva, Yu.V. Kozlov, A.P. Ryskov and G.P. Georgiev, Molekul. Biol. 2 (1968) 736.
[7] R.B. Church and B.J. McCarthy, Biochem. Genet. 2 (1968) 55.
[8] M. Melli and J.D. Bishop, J. Mol. Biol. 40 (1969) 117.
[9] V. Ya. Arion and G.P. Georgiev, Dokl. Akad. Nauk SSSR 172 (1967) 716.